AMENDMENTS TO THE SPECIFICATION

Please amend Examples 19, 20 and 21 on pages 115-117 as follows:

Example 19: Phosphopeptide Detection on Microarrays

Two peptides, Kemptide and pDSIP, were arrayed on to HydroGel coated slides (Perkin Elmer) from a source plate (384-well) with a concentration of 0.03125 to 2 mg/mL peptide in water. The amino acid sequence of Kemptide is ~~Leu-Arg-Arg-Ala-Ser-Leu-Gly~~ LRRASLG (SEQ ID NO. 1, MW 771.9). The amino acid sequence of pDSIP is ~~Trp-Ala-Gly-Gly-Asp-Ala-Ser(PO$_3$H)-Gly-Glu~~ WAGGDASGE (SEQ ID NO. 2, MW 929.5), wherein the serine residue is substituted with PO_2H. Arrays were spotted using a manual glass slide arrayer (V & P Scientific, San Diego, CA) fixed with 4 rows of 8 pins (32 total), ~500 micron diameter spot size, 1.125 micron horizontal pitch and 750 micron vertical pitch (pitch = center to center spacing of spots). The hand arrayer collected 6 nL of peptide from the source plate and transferred ~6 nL to the array surface by direct contact. The resultant peptide concentration was 0.18 to 12 ng/spot. Peptides were arrayed in replicates of 6, resulting in an array of 84 spots. For specific detection of pDSIP, the phosphopeptide, slides were incubated for 1 hour on a rotator in 1 μM dye of compound 2 in buffer containing 0.5 M NaCl, 20% 1,2-propanediol, 1 μM $GaCl_3$, and 0.05 M NaOAc, pH 4.0. Slides were then washed for 1 hour on a rotator in 0.05 M NaOAc, pH 4.0, containing 10% methanol followed by a 15-minute water wash. Slides were then spun briefly in a microarray high-speed centrifuge affixed with a rotor with a slide holder (Telechem) at ~ 6000 rpm to remove excess liquid. After the slides were dry, the arrays were imaged using the ScanArray® 5000 XL Microarray Analysis System (Packard Instrument Co., Meriden, CT) using the 543.5 nm laser and either 570 nm or 592 nm emission filter.

Example 20: Detection of Immobilized Kinase Substrates in Microarray Format; Selective Detection of Glycogen Synthase 1–10.

Two specific peptides, Abl peptide and glycogen synthase 1-10, were arrayed from a source plate (384-well plate) concentration of 0.03 – 2 mg/mL in water, onto HydroGel

coated slides (Perkin Elmer). Abl peptide (New England Biolabs) is a substrate for Abl tyrosine kinase and its amino acid sequence is E-A-I-Y-A-A-P-F-A-K-K-K (SEQ ID NO. 3, MW 1336). Glycogen synthase 1–10 (Calbiochem) is a substrate for Calcium-Calmodulin-Dependent protein Kinase II and its amino acid sequence is P-L-S-R-T-L-S-V-S-S (SEQ ID NO. 4, MW 1045.2). Arrays were spotted using a manual glass slide arrayer (V&P Scientific, San Diego, CA) fixed with 4 rows of 8 pins (32 total), ~500 micron diameter spot size, 1.125 micron horizontal pitch and 750 micron vertical pitch (pitch = center-to-center spacing of spots). The handarrayer collected 6 nL of peptide from the source plate and transferred ~6 nL to the hydrogel coated slide by direct contact. The resultant peptide concentration is 0.18 to 12 ng/spot. Peptides were arrayed in replicates of 6, resulting in array of 96 spots (12 spots, of which were 0 ng/spot). Slides were left overnight after arraying in a humidity chamber. Slides were then blocked for 1 hour in 100 mM HEPES, 1% BSA while rotating (Barnstead/Thermolyne Labquake rotisserie). After blocking, the slides were spun briefly in a small microarray high-speed (max ~6000 rpm) centrifuge affixed with a rotor with a slide holder (Telechem) to remove excess liquid. Next, kinase reactions were performed by attaching a Grace Biolabs Hybriwell™ hybridization sealing system (40 x 22 x 0.25 mm) to the hydrogel coated slide to enclose the area containing the hydrogel polyacrylamide pad. The reaction was carried out in an 80 μL reaction volume containing 20,000 U/mL or 1600 units enzyme (Calmodulin-Dependent protein Kinase II, NEB) using buffer, $CaCl_2$, calmodulin, and ATP supplied with the enzyme. 1X CamKII buffer included 50 mM Tris-HCl, 10 mM $MgCl_2$, 2 mM dithiothreitol, 0.1 mM Na_2EDTA, pH 7.5. $CaCl_2$, calmodulin and ATP working concentrations were 2 mM, 1.2 μM and 0.10 mM. The reaction solution with enzyme was pipetted into the Hybriwell™ through 1 of 2 ports on the seal cover. Ports were then sealed with seal-tabs, placed in a CMT-hybridization chamber (VWR Scientific) and incubated on a nutator (Clay Adams) in a 37°C incubator. The kinase reaction was carried out for 3 hours. After incubation, the slides were removed from the hybridization chamber and washed 2 times for 5 minutes in 10% SDS followed by 5–7 times for 5 minutes in water while rotating. Slides were then transferred immediately to binding solution comprising 1 μM of compound 2 in 50 mM NaOAc, pH 4.0; 500 mM NaCl; 20% 1,2-propanediol; and 1 μM $GaCl_3$ for 45 minutes while rotating. Slides were then washed 3 times for 15 minutes each time in 50 mM NaOAc, pH 4.0, 10% methanol followed by a 15-minute water wash. Slides were then dried and imaged using the Scan Array® 5000 XL Microarray Analysis System (Packard Instrument Co.,

Meriden, CT) using the 543.5 nm laser and either 570 nm or 592 nm emission filters. Calmodulin-dependent kinase II specifically phosphorylates glycogen synthase 1–10 peptide. Using the 543.5 nm excitation and 570 nm emission filter, glycogen synthase peptide is the only fluorescently labeled peptide on the array. Sensitivity of detection after kinase reaction is at least 0.375 ng or 0.35 pmol.

Example 21. Detection of Immobilized Kinase Substrates in Microarray Format; Specific Detection of Abl Peptide Substrate

The following experiment was performed essentially as described in Example 20 with the following differences. Two specific peptides, Abl peptide and Kemptide, were arrayed from a source plate (384-well plate) concentration of 0.03 to 2 mg/mL in water, onto hydrogel-coated slides (Perkin Elmer). Kemptide (New England Biolabs) is a substrate for cAMP-dependent Protein Kinase (PKA) catalytic subunit and its amino acid sequence is L-R-R-A-S-L-G (SEQ ID NO. 5, MW 771). Arrays were spotted as described in Example 20 and kinase reactions performed as described previously. The reaction was carried out in a 80 μL reaction volume containing 3,750 U/mL or 300 units enzyme (Abl Protein Tyrosine Kinase, NEB) using buffer and ATP supplied with the enzyme. 1X Abl buffer included 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM EGTA, 2 mM dithiothreitol, 0.01% Brij 35, pH 7.5. Labeling of slides and imaging were performed as previously described. Using the 543.5 nm excitation and 570 nm emission filter, Abl peptide substrate is the only fluorescently labeled peptide on the array. Sensitivity of detection after kinase reaction is at least 0.18 ng or 0.14 pmol.

Please amend Examples 29 and 30 on pages 124-125 as follows:

Example 29: Detection of phosphopeptides on streptavidin-polystyrene beads using a binding solution of the present invention

Streptavidin-polystyrene beads (4.0-4.9 μM) were charged with either one of two biotinylated synthetic peptides, a phosphopeptide or a non-phosphopeptide. The phosphopeptide had a molecular weight of 1812 g/mol and the amino acid sequence was ~~biotinyl-ε-aminocaproyl-Glu-Pro-Gln-Tyr(PO_3H_2)-Glu-Glu-Ile-Pro-Ile-Tyr-Leu-OH~~

biotinyl-ε-aminocaproyl-EPQYEEIPIYL-OH (SEQ ID NO. 6), wherein the tyrosine residue located between Q and E is substituted with PO_3H_2. The non-phosphopeptide had a molecular weight of 2342.55 g/mol and the amino acid sequence was biotinyl-EGPWLEEEEEAYGWMDF-NH_2 ~~biotinyl-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2~~ (SEQ ID NO. 7). Beads were charged in 100 mM Tris, 100 mM NaCl, pH 7.5 and washed several times in the same buffer, following charging, before staining. Both sets of beads were then stained with 1 μM Compound 2 in buffer containing 0.05 M sodium acetate, pH 4.0, 1 μM $GaCl_3$, 0.5 M NaCl, and 20% 1,2 propanediol for 45 minutes. Following staining, beads were washed in 0.05 M sodium acetate, pH 4.0, 4% acetonitrile and mixed in different ratios of phosphopeptide-charged beads with non-phosphopeptide charged beads. All steps were performed with rotation and rigorous sonication and vortexing. The mixed bead populations were then imaged using a Nikon Eclipse 800 Epi-Fluorescent Microscope using Omega Optical, Inc. filter set XF102-2 (Exciter: 560AF55; Dichroic: 595DRLP; Emitter: 645AF75). The fluorescent signal of the phosphopeptide charged beads was found to be 6-fold higher, on average, than the non-phosphopeptide charged beads.

Example 30: Detection of kinase-mediated phosphorylation of peptide substrates bound to streptavidin-polystyrene particles using a binding solution of the present invention.

Streptavidin-polystyrene beads (4.0-4.9 μM) were charged with a biotinylated synthetic peptide called crosstide. Crosstide is a peptide substrate for the serine/threonine kinase Akt/Protein Kinase B and is a 1808 g/mol peptide with the following amino acid sequence~~, Gly-Arg-Pro-Arg-Thr-Ser-Ser-Phe-Ala-Glu-Gly~~, GRPRTSSFAEG (SEQ ID NO. 8). Beads were charged in 100 mM Tris, 100 mM NaCl, pH 7.5 and washed several times in the same buffer, following charging, before staining. The crosstide peptide on the streptavidin polystyrene particle was then phosphorylated using 500 ng of Akt/PKB kinase in 40 μL of 15 mM MOPS, pH 7.2, 18.75 mM β-glycerol phosphate, 3.75 mM EGTA, 0.75 mM sodium orthovanadate, 0.75 mM DTT supplemented with 200 μM ATP. A control reaction was performed in which all reaction components were added, including ATP, except the kinase enzyme. Phosphorylation was carried out for 60 minutes at 30°C with continuous rotation and stopped by incubating beads and kinase at

100°C for 5 minutes. Beads were then washed again by incubating in 100 mM Tris, 100 mM NaCl, pH 7.5 followed by staining with 1 μM Compound 2 in buffer containing 0.05 M sodium acetate, pH 4.0, 1 μM $GaCl_3$, 0.5 M NaCl, and 20% 1,2 propanediol for 45 minutes. Following staining, beads were washed in 0.05 M sodium acetate, pH 4.0, 4% acetonitrile and imaged using a Nikon Eclipse 800 Epi-Fluorescent Microscope using Omega Optical, Inc. filter set XF102-2 (Exciter: 560AF55; Dichroic: 595DRLP; Emitter: 645AF75). The fluorescent signal of the peptide charged beads exposed to Akt/PKB kinase was found to be 2.2-fold higher, with no overlap in standard deviation, than the control peptide charged beads not exposed to enzyme.